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(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent).

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Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PYRIMIDINE BIOSYNTHESIS INHIBITORS USEFUL AS IMMUNOSUPPRESSIVE AGENTS

(57) Abstract

The pyrimidine biosynthesis inhibitors dichloroallyl lawsone, N-(phosphonoacetyl)-L-aspartic acid (PALA), pyrazofurin, and derivatives thereof, are useful as immunomodulatory and anti-inflammatory agents. Pharmaceutical formulations containing these compounds are useful for the treatment of autoimmune diseases, chronic inflammatory diseases, and of organ transplantation rejections.

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Title

Pyrimidine Biosynthesis Inhibitors
Useful as Immunosuppressive Agents

Background of the Invention

Field of Invention:

This invention relates to methods of treating autoimmune and chronic inflammatory diseases, and organ transplantation rejections and more particularly to such methods using pyrimidine biosynthesis inhibitors.

Prior Art:

A dysfunction of the immune system can manifest itself as an autoimmune disease such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematous, and the like, and chronic inflammatory diseases. Organ transplantation rejection may also be an immune-based inflammatory response. Agents which have an immunosuppressive effect would be highly desirable for the treatment of these diseases.

Information Disclosure:

Dichloroallyl lawsone is an anticancer drug which is described in U.S. Patent 3,655,699, granted April 11, 1972, to H. Putner.

Pyrazofurin and pyrazofurin B are antibiotics having antiviral and antifungal activity. These compounds, their alkanoyl derivatives, and their preparation are described in U.S. Patents 3,802,999, granted April 9, 1974, to Williams et al.; U.S. 3,998,999, granted December 21, 1976, to De Bernardo et al.; and U.S. 3,960,836, granted June 1, 1976, to Gatowski.

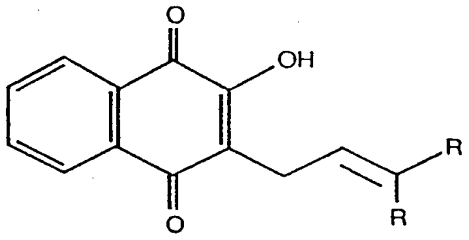
N-(phosphonoacetyl)-L-aspartic acid (PALA) and its analogs are compounds useful for the treatment of cancer. These compounds, intermediates thereto and

their preparation are described in U.S. Patents 4,267,126, granted May 12, 1981, to Schultz et al.; U.S. 4,215,070, granted July 29, 1980, to Schultz et al.; U.S. 4,179,464, granted December 18, 1979, to Schultz et al., U.S. 4,154,759, granted May 15, 1979, to Parsons; and U.S. 4,178,306, granted December 11, 1979, to Parsons.

Summary of the Invention

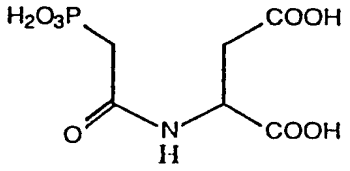
According to the present invention there is provided a method of treating an autoimmune disease, a chronic inflammatory disease, or organ transplantation rejection in a mammal comprising administering to the mammal an effective amount of a pyrimidine biosynthesis inhibitor selected from the group consisting of:

(a) a compound of the formula:



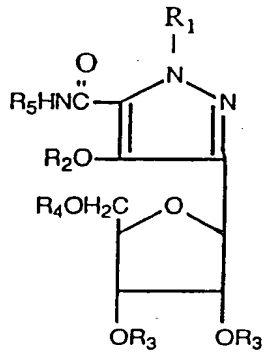
wherein each R is CF_3 or halogen;

(b) a compound of the formula



or a pharmaceutically acceptable salt thereof, or dialkyl (C_1-C_4) or dibenzyl ester thereof; and

(c) pyrazofurin of the formula:



where R_1, R_2, R_3 and R_5 independently are H or C_1-C_6 alkanoyl and R_4 is H, C_1-C_6 alkanoyl, palmitoyl, benzoyl, or adamantoyl.

Detailed Description of the Invention

The compounds useful in the method of this invention are known compounds described in the U.S. patents set forth in the Information Disclosure section, supra. The disclosures in these patents to the compounds and their preparation are hereby incorporated by reference.

Preferred compounds are (1) those of formula (a) wherein each R is halogen, particularly Cl; (2) those of formula (b) wherein a salt of the compound is used, particularly an alkali metal salt; and (3) those of formula (c) wherein each of R_1-R_5 is H.

The specifically preferred compounds useful in the present method are dichloroallyl lawsone, PALA, disodium

salt and pyrazofurin. These are specific compounds described in the aforesaid patents.

The invention can be further understood by the following examples in which parts and percentages are by weight unless otherwise indicated.

Human Mixed Lymphocyte Reaction

Blood was obtained by venipuncture from two nonrelated human donors. Peripheral blood mononuclear cells (PBMC) were isolated from these samples by using the Leuco Prep procedure (Becton-Dickinson). PBMC were washed twice in phosphate buffered saline (without calcium and magnesium) and the separate cell isolations were adjusted to the appropriate concentrations in media (RPMI 1640) supplemented with 10% human AB serum and 50 ul/ml gentamicin. Cells from donor A (2×10^5) were incubated with cells from donor B (2×10^5) with or without compound in 96 well round bottom microtiter plates at 37°C, 5% CO_2 for 6 days. Eighteen hours prior to harvesting cells from the plates, all wells were pulsed with 1 uCi of tritiated-thymidine. Cells from the plates were harvested on day 6 and tritiated-thymidine incorporation was determined using a scintillation counter. Test results are shown in the following table.

COMPOUND	IC50(M)
Pyrazofurin	8.0×10^{-9}
Dichloroallyl Lawsone	4.5×10^{-6}
PALA	4.5×10^{-5}

The test results show that these compounds suppress an in vitro immune response. Based on these data, the compounds useful in this invention should be efficacious in treating autoimmune diseases, multiple sclerosis and

chronic inflammatory diseases such as rheumatoid arthritis; all of which involve T lymphocyte mediated components. Activities in the human mixed lymphocyte reaction indicate that the compounds useful in the invention should be effective in preventing transplantation rejection and graft vs. host disease.

DOSAGE FORMS

The useful compounds (active ingredients) of this invention can be administered by any means that produces contact of the active ingredient with the agent's site of action in the body of a mammal. They can be administered by any conventional means available for use in conjunction with pharmaceuticals; either as individual therapeutic active ingredients or in a combination of therapeutic active ingredients. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

The dosage administered will be an effective amount of active ingredient and will, of course, vary depending upon known factors such as the pharmacodynamic characteristics of the particular active ingredient, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms; kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a daily dosage of active ingredient per kilogram of body weight. Ordinarily 1 to 100, and preferably 10 to 50 milligrams per kilogram per day is effective to obtain desired results.

Dosage forms (compositions) suitable for internal administration contain from about 10-500 milligrams to about 500 milligrams of active ingredient per unit. In

these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-95% by weight based on the total weight of the composition.

The active ingredient can be administered orally in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions, it can also be administered parenterally, in sterile liquid dosage forms.

The active ingredient can be administered orally in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions, it can also be administered parenterally, in sterile liquid dosage forms.

Gelatin capsules contain the active ingredient and powdered carriers, such as lactose, sucrose, mannitol, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract.

Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

In general, water, a suitable oil, saline, aqueous dextrose (glucose), an related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration contain preferably a water soluble salt of the active

ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents such as sodium bisulfite, sodium sulfite, or ascorbic acid either alone or combined are suitable stabilizing agents. Also used are citric acid and its salts and sodium EDTA. In addition parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

CAPSULES

A large number of unit capsules are prepared by filling standard two-piece hard gelatin capsules each with 100 milligrams of powdered active ingredient, 175 milligrams of lactose, 24 milligrams of talc, and 6 milligrams magnesium stearate.

A mixture of active ingredient in soybean oil is prepared and injected by means of a positive displacement pump into gelatin to form soft gelatin capsules containing 100 milligrams of the active ingredient. The capsules are washed and dried.

TABLETS

A large number of tablets are prepared by conventional procedures so that the dosage unit is 100 milligrams of active ingredient, 0.2 milligrams of colloidal silicon dioxide, 5 milligrams of magnesium stearate, 275 milligrams of microcrystalline cellulose, 11 milligrams of cornstarch and 98.8 milligrams of lactose. Appropriate coatings may be applied to increase palatability or delay absorption.

INJECTABLE

A parenteral composition suitable for administration by injection is prepared by stirring 1.5%

by weight of active ingredient in 10% by volume propylene glycol and water. The solution is made isotonic with sodium chloride and sterilized.

SUSPENSION

An aqueous suspension is prepared for oral administration so that each 5 milliliters contain 100 milligrams of finely divided active ingredient, 200 milligrams of sodium carboxymethyl cellulose, 5 milligrams of sodium benzoate, 1.0 grams of sorbitol solution, U.S.P., and 0.025 milliliters of vanillin.

The same dosage forms can generally be used when the compounds of this invention are administered stepwise in conjunction with another therapeutic agent. When the drugs are administered in physical combination, the dosage form and administration route should be selected for compatibility with both drugs. Suitable dosages, dosage forms and administration routes are illustrated in the following table.

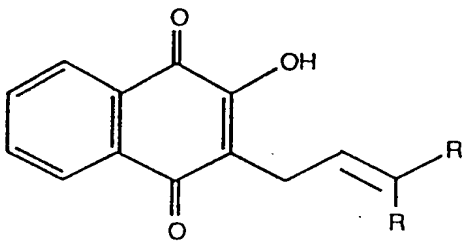
Examples of NSAID's that can be combined with the compounds used in this invention:

Drug	Dose (mg)	Formulation	Route
Indomethacin	25 (2/3 times daily)	Tablet	Oral
Meclofenamate	50-100 (2/3 times daily)	Tablet	Oral
Ibuprofen	300-400 (3/4 times daily)	Tablet	Oral
Piroxicam	10-20 (1/2 times daily)	Tablet	Oral
Sulindac	150-200 (1/2 times daily)	Tablet	Oral
Azapropazone	200-500 (3/4 times daily)	Tablet	Oral

WHAT IS CLAIMED IS:

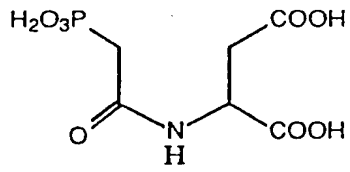
1. A method of treating an autoimmune disease, a chronic inflammatory disease, or organ transplantation rejection in a mammal comprising administering to the mammal an effective amount of a pyrimidine biosynthesis inhibitor selected from the group consisting of:

(a) a compound of the formula:



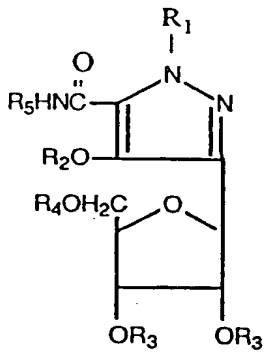
wherein each R is CF_3 or halogen;

(b) a compound of the formula



or a pharmaceutically acceptable salt thereof, or dialkyl or dibenzyl ester thereof; and

(c) pyrazofurin of the formula:



where R_1, R_2, R_3 and R_5 independently are H or C_1-C_6 alkanoyl and R_4 is H, C_1-C_6 alkanoyl, palmitoyl, benzoyl, or adamantoyl.

2. The method of Claim 1 wherein the compound is dichloroallyl lawsone.

3. The method of Claim 1 wherein the compound is N-(phosphonoacetyl)-L-aspartic acid, disodium salt.

4. The method of Claim 1 wherein the compound is pyrazofurin.

5. The method of Claim 1 wherein the compound is administered in combination with a nonsteroidal antiinflammatory drug.

6. The method of Claim 2 wherein the compound is administered in combination with a nonsteroidal antiinflammatory drug.

7. The method of Claim 3 wherein the compound is administered in combination with a nonsteroidal antiinflammatory drug.

8. The method of Claim 4 wherein the compound is administered in combination with a nonsteroidal antiinflammatory drug.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05942

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5)G01N33/544; C12N11/02; G01N33/553; G01N33/549, C07K17/02; A61K9/00

USC1: 514/75, 407, 732, 825, 885, 903; 548/374

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
US	514/75, 732, 825, 885. 903, 407; 548/374

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EP, A, 0,189,019 (U.W. SLEYTR et al) 30 July 1986, See entire document.	1-8
A	EP, A, 0,154,620 (U.W. SLEYTR et al) 01 January 1986. See entire document.	1-8
A	EP, A, 0,173,500 (Decan et al) 05 March 1986. See entire document.	1-8
A	EP, A, 0,184,710 (Muller-Ruchhultz et al) 18 June 1986. See entire document.	1-8
A	US, A, 3,979,184 (I. GIAEVER) 07 September 1976. See entire document.	1-8
A	EP, A, 0,166,233 (Keck et al) 02 January 1986. See entire document.	1-8
A	US, A, 4,788,061 (SHORE) 29 November 1988. See entire document.	1-8

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
08 January 1991	11 MAR 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Ronald W. Griffin

Form PCT/ISA/210 (second sheet) (May 1986)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No **
A	US, A, 3,655,699 (RUTNER) 11 April 1972. See entire document.	1-8
A	US, A, 3.802.999(WILLIAMS ET AL)09 April 1974. See entire document.	1-8
A	US, A, 3,960,836 (GUTOWSKI) 01 June 1976. See entire document.	1-8
A	US, A, 3,998,999 (DE BERNARDO ET AL) 21 December 1976. See entire document.	1-8
A	US, A, 4,215,070 (SCHULTZ ET AL) 29 July 1980. See entire document.	1-8
A	US, A, 3,957,836 (MORIMOTO ET AL) 18 May 1976. See entire document.	1-8
A	US, A, 4,092,414 (CRAGOE ET AL) 30 May 1978. See entire document.	1-8

International Application No. PCT/US90/05942

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..... . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. X OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

The following are independent and distinct species pertinent to the pyrimidine biosynthesis inhibitor employed to treat an autoimmune disease, a chronic imflammatory disease or organ transplantation rejection in a mammal where (a) is the first species and will be searched with claims 1, 2, 5, 6, 7 and 8 in the event that no other fees are paid. Note that a search of any other additional species requires payment of additional fees. See Attachment.

1. X As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.